

Amendments to the Specification

Please amend the specification as follows:

At the Title:

Method and Apparatus for ~~Counterconcurrent~~ Countercurrent Chromatography

SS 11/22/10 At paragraph [000⁵~~7~~]:

Some countercurrent chromatography systems utilize a complex hydrodynamic motion in two solvent phases within a column comprising a rotating coiled tube. If, for example, a horizontally mounted coil is filled with water and is rotated around its own axis, any object, either heavier or lighter than the water present in the column will tend to move toward one end of the coil. This end is then called the "head" and the other end, the "tail" of the coil. When the coil is filled with two immiscible solvent phases, the rotation establishes a hydrodynamic equilibrium between the two solvent phases, where the two phases are distributed in each turn at a given volume ratio (equilibrium volume ratio) and any excess of either phase remains at ~~either the head or the tail of the coil for each solvent respectively.~~

SS 11/22/10 At paragraph [000⁶~~8~~]:

When one of the solvents phases is added to the coil ~~from the~~ at its tail end and is further eluted from the coil ~~from~~ at its head end, the hydrodynamic equilibrium tends to maintain the original equilibrium volume ratio of the two phases in the coil and thereby a certain volume of the other phase is permanently retained in the coil while the two phases are undergoing vigorous agitation with rotation of the coil. As a result, the sample solutes present in one phase and introduced locally at the inlet of the coil are subjected to an efficient partition process between the two phases and are chromatographically separated according to their partition coefficients.

SS 11/22/10 At paragraph [002²²~~4~~]:

Although embodiments of the invention have various applications, many advantageous embodiments of the present invention are directed to an improved ~~column~~ apparatus for use in countercurrent chromatography. Applicable chromatography techniques include those using synchronous planetary motion such as X-type, J-type, and I-type chromatography. The apparatus and methods described herein are especially advantageous when applied to high-speed countercurrent chromatography (HSCCC) with high flow rates. The column design may also be employed in large column applications for industrial-scale separations of samples by mounting the column assembly on a slowly rotating horizontal shaft. Some aspects of the invention are based, in part, on the surprising discovery that the retention of the stationary phase is improved if the configuration of the column used in ~~counter-current~~ countercurrent chromatography is modified from coiled tubing to a series of grooved plates (also referred to herein as separation disks) forming a plurality of interleaved spiral channels. The centrifugal force gradient produced by the spiral pitch in the separation disks helps to more efficiently distribute the heavier phase in the periphery and the lighter phase in the proximal portion of the column.

SS 11/22/10 At paragraph [0023]:

This centrifugal force effect on a sample is enhanced by increasing the pitch of the spiral, and a spiral column assembly prepared by simply winding tubing into a flat spiral configuration like thread on a spool provides only a limited spiral pitch. In accordance with these observations, a column for use in high speed ~~counter-current~~ countercurrent chromatography has been developed having interleaving, grooved separation disks with multiple flow channels instead of coiled tubing. The use of a column having interleaving, grooved separation disks has many advantages over the prior art. First, a column possessing a plurality of interleaving, grooved separation disks with multiple flow channels provides a greater spiral pitch than previous column designs, facilitating the movement of a fluid sample radially outward at a faster rate than with previous designs, thereby providing a more efficient separation of the two phases of a fluid sample. Finally, the column design of the present invention obviates the shortcomings of earlier column designs inasmuch as it is easier to manufacture.

§ 4/22/10 At paragraph [0028]²⁶:

Still with reference to **FIG. 1**, ~~a pair of flow tubes~~ an inlet flow tube 24 ; and an outlet flow tube 26 ~~are led~~ may pass through a center ~~hole~~ bore in the column holder shaft **20** downward and out of the column assembly 10 to exit the complete centrifuge apparatus (not shown), for example at a center hole in an upper plate of the ~~whole~~ complete centrifuge assembly apparatus ~~where~~ and the flow tubes inlet flow tube 24 ; and outlet flow tube 26 may be tightly fixed with a pair of clamps (see, for example, the routing shown in U.S. Pat. No. 4,430,216). Advantageously, ~~these~~ flow tubes **24** and **26** may be protected with a sheath of flexible tubing such as Tygone (Norton Company, Worcester, Mass.) to prevent direct contact with metal parts in the complete centrifuge apparatus assembly.

§ 4/22/10 At paragraph [0029]²⁷:

FIG. 2 is an exploded view of the components of the column assembly illustrated in **FIG. 1**. The column assembly **10** comprises an upper flange **12** having a gear **22** and a lower flange **14** . Disposed between the upper flange **12** and the lower flange **14** is are a plurality of ~~coupled~~ fluidly connected separation disks **16a-16b**. Alternating between each of the plurality of separation disks **16a-16b** is a septum **18a** . Another septum 18b is situated between the upper flange 12 and the uppermost separation disk 16a, and yet another septum 18c is situated between the lowermost separation disk 16b and the lower flange 14. The separation disks **16** can be constructed from stainless steel or a plastic such as a PTFE, high density polyethylene, or any other suitable polymer. Advantageously, the separation disk **16** may have a diameter of between 1 cm and 30 cm, and a thickness of between 0.5 and 20 mm. In one embodiment that has been found suitable, the separation disk **16** has a diameter of about 17.5 cm and a thickness of about 4 mm. Preferably, the septum **18** is constructed of PTFE (e.g. Teflon®, E.I. Du Pont, Wilmington, Del.). One of skill in the art would appreciate that the septum **18** can be constructed from any number of suitable non-reactive materials. The construction of the separation disks **16** and septa **18** are described in additional detail below with reference to **FIGS. 3, 4A, and 4B**.

At paragraph [0030]:

Turning now to **FIG. 3**, a first embodiment of a separation disk **16** having a first surface **30** and a second opposed surface **32** is illustrated. The separation disk **16** includes an inner edge **40** and an outer edge **42**. The separation disk **16** comprises a single spiral flow channel **44** carved, etched, or molded on the surface of the first side **30** of the separation disk **16**. The spiral flow channel **44** has an inlet end **46** and an outlet end **48** with fluid flow typically traveling along the path of the spiral channel **44** from the inlet end **46** to the outlet end **48**. Advantageously, the spiral channel **44** of one separation disk **16** is serially connected to the spiral channel 44a of another separation disk 16a (not shown) by stacking multiple separation disks **16** adjacent to one another with a septum septa 18 separating each pair. Preferably, the outlet end **48** of the channel **44** connects to the inlet end 46a of the channel 44a on the next adjacent disk 16a (not shown). To accomplish this, the bottom of the outlet end **48** of the channel **44** includes a hole an outer hole 62 (which may be about 1 mm diameter) that is connected to a radial channel 49 grooved or molded into the ~~other side~~ second opposed surface 32 of the separation disk **16**, with a hole (which may be about 1 mm diameter) that extends through the thickness of the separation disk **16**. The radial channel 49 ~~on~~ is grooved within the other side of the disk second opposed surface 32 extends radially inward and until it is substantially aligned aligns with the inlet end **46** of the spiral channel **44** and is physically separated from the inlet end **46** by the material of the separation disk **16**. An inner end hole 64 of in the radial channel 49 is adjacent to a hole in the septum **18** (not shown) that connects to the inlet end 46a of the spiral channel 44a on the next adjacent disk 16a (not shown).

At paragraph [0031]:

To hold the separation disks **16** together in making the whole column assembly 10, ~~the each~~ separation disk **16** advantageously includes a plurality of screw holes **50** at regular intervals at near both the inner **40** and outer **42** edges of the separation disk **16**. In some suitable embodiments, the screw holes **50** are positioned circumferentially at approximately 10 degrees spacings for the outer edge **42** and 45 degrees spacings for

the inner edge **40**. Similar holes (not shown) are also made in both the septa **18** and the flanges **12 and 14** as will be described in greater detail with reference to **FIGS. 5, 6, and 7**.

At paragraph [0030]:

In a preferred embodiment, multiple interleaved spiral flow channels are incorporated symmetrically around the center of a separation disk **16** so that the spiral pitch is increased as compared to the spiral pitch of a single spiral channel **44** such as is shown in **FIG. 3**. **FIG. 4A** is a top view of the first surface **30** of a separation disk **16** having a plurality of interleaved spiral channels **52, 54, 56, and 58**. **FIG. 4B** is a cross section of the separation disk **16** of **FIG. 4A**, and **FIG. 4C** is a top plan view of the separation disk **16** of **FIGS. 4A and 4B**. As illustrated in these Figures, the separation disk **16** has four interconnected separate spiral channels **52, 54, 56, and 58**, respectively. However, it will be appreciated that the number of spiral channels can vary. Each channel may be between 0.25 mm and 10 mm wide. Preferably, the width of each channel may be between 0.5 mm and 7 mm wide, with 3 mm having been found suitable in one embodiment. The depth of each channel can likewise vary. Preferably, the depth of each channel is between 0.1 mm and 5 mm, with 2 mm having been found suitable in one embodiment. Depending on the size of the disk, each channel may have a length of between about 250 mm and 5 m. In one advantageous embodiment, the length of each channel is approximately 1 m. It will be appreciated, however, that the length of each channel can vary. Each groove or channel is separated from the next groove or channel by a ridge **60**, which may measure approximately 1 mm in width for 3 mm wide channels.

At paragraph [0031]:

Still with reference to **FIG. 4A**, each channel **52, 54, 56, and 58** has an inner end denoted **I1, I2, I3, and I4** respectively in **FIG. 4A**. The channels **52, 54, 56, and 58** each begin at their inner ends **I1, I2, I3, and I4** and spiral around to their outer ends denoted **O1, O2, O3, and O4** respectively in **FIG. 4A**. In the embodiment of **FIG. 3B-FIG. 4A**, each channel **52, 54, 56, and 58** forms 3.25 2.75 spiral turns so that the outer end of a

given channel is at the same angular location relative to the inner end of the next channel. Thus, as shown in **FIG. 4A**, **O1** is at the same angular orientation as **I2**, **O2** is at the same angular orientation as **I3**, ~~etc.~~ and **O3** is at the same angular orientation as **I4**.

11/22/10 At paragraph [0034]³²:

Connection between ~~an~~ the outer outlet end of one spiral channel **O1**, **O2**, and **O3** and ~~an~~ the inlet inner end **I2**, **I3**, and **I4**, respectively of the next spiral channel is made by ~~another~~ connecting channels **72**, **76**, and **78**, respectively, that are formed on the second opposed surface **32** of the separation disk **16** as illustrated with dashed lines on **FIG. 4A** and also on **FIG. 4BC**, which shows a top plan view of the second opposed surface **32**. Except for the ~~inlet~~ inner end **I1** of the first channel **52** ~~(I1)~~ and the outer end **O4** of the last channel **58**, each end of the channels **52**, **54**, **56**, and **58**, have a hole **70** (approximately 1 mm in diameter) through the separation disk **16** which communicates with the end of a the connecting channels **72**, **76**, or **78** on the ~~opposite~~ opposed surface **32** of the separation disk **16**.

11/22/10 At paragraph [0035]³³:

Referring now to **FIGS. 4A, 4B, 4C**, and **5**, when a sample solution is introduced into the column assembly **10**, fluid enters the inner end **I1** of the first channel **52** and travels in the direction of the spiral to the outer end **O1** of the first channel **52**. At this point, the fluid travels through hole **70** to the end of the linear connecting channel **72** on the ~~other side of the disk~~ opposed surface 32 of the separation disk **16**, thereby providing a flow path for the sample to travel from the outer end **O1** of the first channel **52** to the radial location of the inner end **I2** of the next channel **54**. From there the fluid flows through another hole **70** ~~extending between the inner end of the linear connecting channel 72~~ and the inner end **I2** of the second spiral channel **54**. The fluid then travels around to the outer end **O2** of the second channel **54**, where it passes through a hole **70** to the connecting radially extending channel 78 on the ~~other side of the disk~~ opposed surface 32 of the separation disk **16**, from where it travels radially to the location of the inner end **I3** of the third channel **56**. This process is repeated through the third ~~and fourth~~

channels— channel 56, connecting channel 78, and fourth channel 58 until the fluid ends up emerges at the outer end O4 of the fourth channel 58. At this point, the fluid passes through another hole 70 at the outer end O4 of the fourth channel 58 and through radial connecting channel 80 to the radial position of inner end I1 where the flow started. However, no hole through the separation disk 16 to the inner end I1 of the first channel 52 is provided. Rather, an interdisk connection hole 84 in the septum 18 is positioned to coincide with the location of the inner end I1 of the ~~flow~~ first channel 80 52 on one separation disk 16 and with the inner end I1a of the first channel 52a on the next adjacent separation disk 16a (not shown). The interdisk connection hole 84 conducts the fluid from the connecting channel 80 of the separation disk 16 to the inner end I1a of the next adjacent separation disk 16a (not shown). Thus, in a series of adjacent separation disks 16a-16b as shown in **FIG. 1**, fluid passes from the outer end O4 of the fourth channel 58 on one separation disk 16a to the beginning inner end I1a of the first channel 52a on the next separation disk 16b (not shown).

At paragraph [0036]³⁴:

In operation, the pitch of each additional spiral on a separation disk 16 may increase markedly as compared to the pitch of a single spiral channel, assuming that the volume of fluid contained in the single spiral channel is essentially the same as the volume of fluid contained in the multiple spirals. For example, when a separation disk 16 with a diameter of about 17.5 cm includes four spiral channels, the pitch ~~can~~ of each spiral may become as large as 16 mm (three times that of the single spiral channel).

At paragraph [0037]³⁵:

One embodiment of the flanges which are placed on the top and bottom of the stack of separation disks 16 of **FIG. 1** is shown in **FIGS. 6** and **7**. The upper flange 12 (**FIG. 6**) is equipped with a gear 22 which engages with a stationary gear on the HSCCC centrifuge (not shown). **FIG. 7** depicts the lower flange 14 which has two screw holes 90, 92 positioned substantially 90 degrees apart circumferentially for tightly fixing the column assembly 10 against the column holder shaft (0.9 inch diameter). Both the upper and lower flanges 12, 14 each have an inlet/outlet hole 94 which fits to an adapter (not

shown) with a screw thread. They also have a set of screw holes **60 66** located at regular intervals around the circumference near the outer and inner edges of each septum 18 ~~as in~~ in alignment with the screw holes 50 of the separation disks 16 and Teflon-septa 18. In preferred embodiments, the screw holes **60 66** are positioned circumferentially at approximately 10 degrees spacings apart for the outer edge and 45 degrees spacings apart for the inner edge.

DN/22/10 At paragraph [0036]³⁶:

It has been observed that the use of a rectangular spiral channel embedded in a solid separation disk **16** as described above enhances the retention of the stationary phase for viscous, low interfacial tension two-phase solvent systems. Accordingly, in one embodiment, a column assembly 10 having a separation disk **16** comprising at least one rectangular spiral channel **44** embedded in the separation disk **16** is provided. The rectangular spiral channel configuration has a number of advantages over the prior art. For example, the rectangular spiral channel is useful for separating biopolymers such as proteins, DNA, RNA, polysaccharides, and cell particles. Additionally, this channel design ensures reliable retention of the stationary phase for polar or low interfacial tension solvent systems such as the 1-butanol/water system to separate bioactive compounds including peptides. Similarly, the rectangular design provides an improved stationary phase retention against emulsification.